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Research Contract DA49-007-MD-317
between the Medical Research and Development Board,
Office of the Surgeon General, Department of the Army,
and the President and Fellows of Harvard College.

Final Report on Contract, Covering All Work Done Under Contract

Contract Dates 1 June 1952 to 31 August 1954

TREATMENT OF WATER BY FLOTATION

42-p

Responsible Investigator: Edward W. Moore, Associate Professor of Sanitary Chemistry

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DATE PROCESSED: 8 March 65

PROCESSOR: Clay

I. Brief Summary of Findings under Contract.

Bacteria, amoebic cysts, and staphylococcus phage particles used as virus simulants, were effectively removed from distilled water suspensions under laboratory conditions, by aerating those suspensions for five to ten minutes with fine bubbles, after addition of 5 or 10 mg/l of several quaternary ammonium compounds. The organisms were presumably either killed by the disinfecting action of the quaternaries, or removed in the foam of air bubbles coming off the suspensions or both. The procedure seemed to offer promise as a means for rapid purification of water with simple equipment in the field. Further investigation showed that the removal was adversely affected by lowering the temperature of the water. It was also adversely affected by several components present in natural waters: those identified in the time available for research were (a) the hardness ions, Ca^{++} and Mg^{++} (b) trivalent ions such as Al^{+++} and presumably Fe^{+++} (c) organic color compounds and presumably other organic matter (d) turbidity when present along with factor (a). The presence of other unidentified interferences is indicated. The adverse effects are roughly additive, so that a highly colored, hard natural water at a low temperature might require 50 or 100 mg/l of quaternary, and aeration for a much longer period, to arrive at a satisfactory degree of purification. Electrophoretic experiments showed that the addition of quaternaries to distilled water suspensions of coliform organisms produced a reversal in sign of charge--from negative to positive--in the organisms, at very low quaternary dosages. When any of the adverse factors were present, this reversal in sign required very much greater amounts of quaternary. The presumption is that the adverse factors interfere in various ways with the sorption of quaternary by the organism.

Since the process proved so sensitive to conditions and water components likely to be encountered in the field, it was concluded that further investigation of the process as a field water-purification method was unwarranted at present. If means can be found for overcoming the adverse effects encountered in natural waters, this conclusion would, of course, be altered.

II. Background and Purpose of Research.

This research project was initiated to investigate the possibility of purifying water by flotation with quaternary ammonium compounds and air, as originally suggested by Professor S. H. Hopper of Indiana University. Since the process conceivably could be adapted to water purification for military installations in the field, a research program was sponsored by the Office of the Surgeon General, Department of the Army. The purpose of this research was to investigate in detail the effectiveness of the process in removing the various classes of pathogens, namely bacteria, amoebic cysts, and viruses from contaminated

waters. If encouraging results were obtained the contractors were to proceed with the research necessary to adapt the process for use in the field.

III. Work Covered in this Report.

The results obtained for the entire duration of the contract from 1 June 1952 to 31 August 1954, are presented in condensed form in this report.

IV. Outline of Experimental Methods.

Suspensions of known concentration of the organisms studied - coliform bacteria, cysts of Entamoeba histolytica, and Staphylococcus phage - in distilled, synthetic, and natural waters were placed in aeration vessels made from Sedgwick-Rafter funnels. The desired quantities of quaternary ammonium compounds were added. The preparations were then aerated for a specified time and at a specified rate, using porous alundum gas dispersers. The foam formed was removed from the top of the vessel. In certain experiments, addition of quaternary and aeration was repeated a second time. The numbers of the specific organisms remaining were determined by appropriate techniques: direct plating, lactose tubes or millipore filters for the coliforms; centrifuging, microscopic count and culturing in appropriate medium for amoebic cysts; and addition to staphylococcus plate cultures for the phage.

The list of quaternary ammonium compounds tried is given below:

1. Hyamine 1622 . Diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride
2. Zephiran . Alkyl dimethyl benzyl ammonium chloride
3. Bradosol . Alkyl dimethyl benzyl ammonium chloride
4. Emulsept . N-(acylcolaminoformyl methyl) pyridinium chloride
5. Arquad T . Dialkyl dimethyl ammonium chloride
6. Ceepryn . Cetyl pyridinium chloride

V. Removal of Coliform Organisms from Distilled Water Suspensions.

Several hundred experiments were run on coliform suspensions in distilled water. The range of initial coliform numbers was from 35×10^6 to 80×10^6 organisms per 100 ml of water; within this range, initial numbers were shown to have no significant effect on the numbers remaining after treatment. With each experiment, a control was run which received the same quaternary dosage but no aeration. The control can be considered to represent direct kill of the organisms by the quaternary, as distinguished from physical removal in the froth. Reduction of coliform numbers in the controls were substantial, but nearly always less than that secured by aeration with the quaternary and removal of foam.

Experiments were run at three temperature ranges: 7-10°C, 20-22°C, and 37°C. A detailed tabulation of these experiments was given on pp. 2-3 of Annual Report dated 31 March 1953, and further data on pp. 6-7 of Annual Report dated 1 April 1954.

The findings were that at 37°C and at 20-22°C, amounts of quaternary not exceeding 5 to 10 mg/l, followed by 5 to 10 minute aeration, sufficed to reduce the initial coliform numbers cited above, to levels of 50 organisms per 100 ml of water or less; - in other words, to produce water of sufficient purity to be safe for drinking if given nominal chlorination, according to U.S.P.H.S. standards. At 7-10°C the quaternary requirement was increased to 20 mg/l to produce a water of the same quality. Arquad T and Ceepryn appeared to be the best of the quaternaries for coliform removal; this correlated roughly with the observed ability of the quaternary to produce a stable foam.

Figure I illustrates some of the data obtained for Arquad T, and shows the very considerable temperature effect.

VI. Removal of E. histolytica Cysts from Suspensions in Distilled Water.

Cyst suspensions made up to contain about 30 to 45 cysts per ml were treated with quaternaries and aerated in the same manner as the coliform suspensions. Similar control tests were made. The treated samples were neutralized with aerosol (10 parts to 1 part of added quaternary) and centrifuged. The centrifugate was counted directly under the microscope, and also incubated in modified Cleveland's medium to determine viability of the cysts. Because of the presence of considerable numbers of starch grains in the cyst suspension the test solution showed an appreciable turbidity. This turbidity was removed during the aeration process. Most of the experiments were at room temperature, 20-23°C; a few were made at 7-10°C.

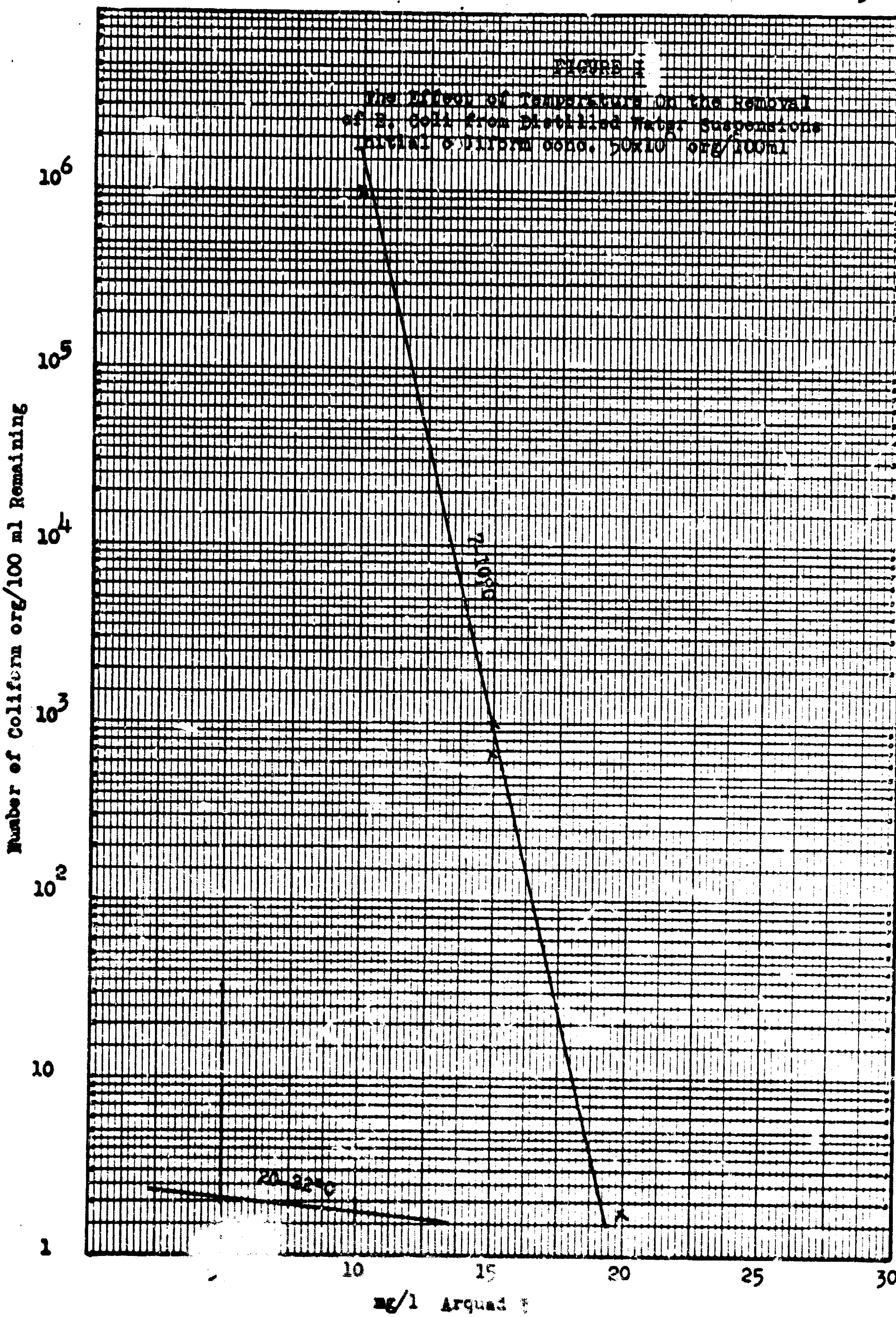
FIGURE I
The Effect of Temperature On the Removal
of E. Coli From Distilled Water Suspensions
Initial coliform conc. 50x10 org/100ml
Number of Coliform org/100 ml Remaining
10^6
10^5
10^4
10^3
10^2
10
1
10
15
20
25
30
mg/l Arquad

Tabulations of these experiments appear on page 4 of Annual Report dated 31 March 53 and page 6 of Quarterly Progress Report dated 31 December 52 and summarized in Table 1 of this report. These data show complete removal of cysts by doses of 5-10 mg/liter of the better of the quaternaries at temperature of 20-23°C and 7-10°C.

Other tests, particularly single treatments with 5 mg/l of quaternary showed cysts remaining in the treated suspension, usually in numbers ranging from 10 to 100 in the entire 500 ml, but occasionally as high as 2000 cysts per 500 ml. These were all negative when tested for viability, except in two experiments; a positive result was secured from a sample singly treated with 5 mg/l Hyamine at 23°C and a positive result was also obtained from a sample singly treated with 5 mg/l Zephiran at 7-10°C. Control tests, using the quaternary ammonium compounds without aeration, and aeration without quaternary, were in all cases found to contain viable cysts in large numbers. Cysts were recovered in large numbers from the foam produced in the tests, and in most cases these cysts were viable, showing that mechanical transfer is important in the removal of cysts from the water rather than the killing action of the quaternary. By contrast, viable coliform organisms were never recovered from the foam in the coliform experiments.

From these results, it was concluded that dependable removal of cysts from distilled water suspensions could be secured by double treatment with 5 mg/l of any of the quaternaries studied at 20-23°C, and with most of them at 7-10°C.

VII. Removal of Staphylococcus Phage from Distilled Water Suspensions.

The work on staphylococcus phage was undertaken to provide an indication of the probable removals of pathogenic virus. Previous work by S. L. Chang has shown that the phage responds to disinfecting agents in the same way as Theiler's virus. Furthermore, the particle size is comparable. A large number of experiments on the removal of staphylococcus phage from suspensions in distilled water were made. Quantities of 20 mg/l of the best of the quaternaries used gave up to 99.8% removal of the phage at room (20-23°C) temperatures from suspensions containing initially from 150,000 to 300,000 particles per ml.

Experiments were also made at temperatures of 7-12°C and 37°C. They showed that the removal is adversely affected by low temperatures and greatly improved at high temperatures.

A series of experiments were made with an anionic (Nacconol) and a non-ionic (Tween 80) detergent. These experiments showed that there was little or no removal of phage in either case although copious foaming was observed.

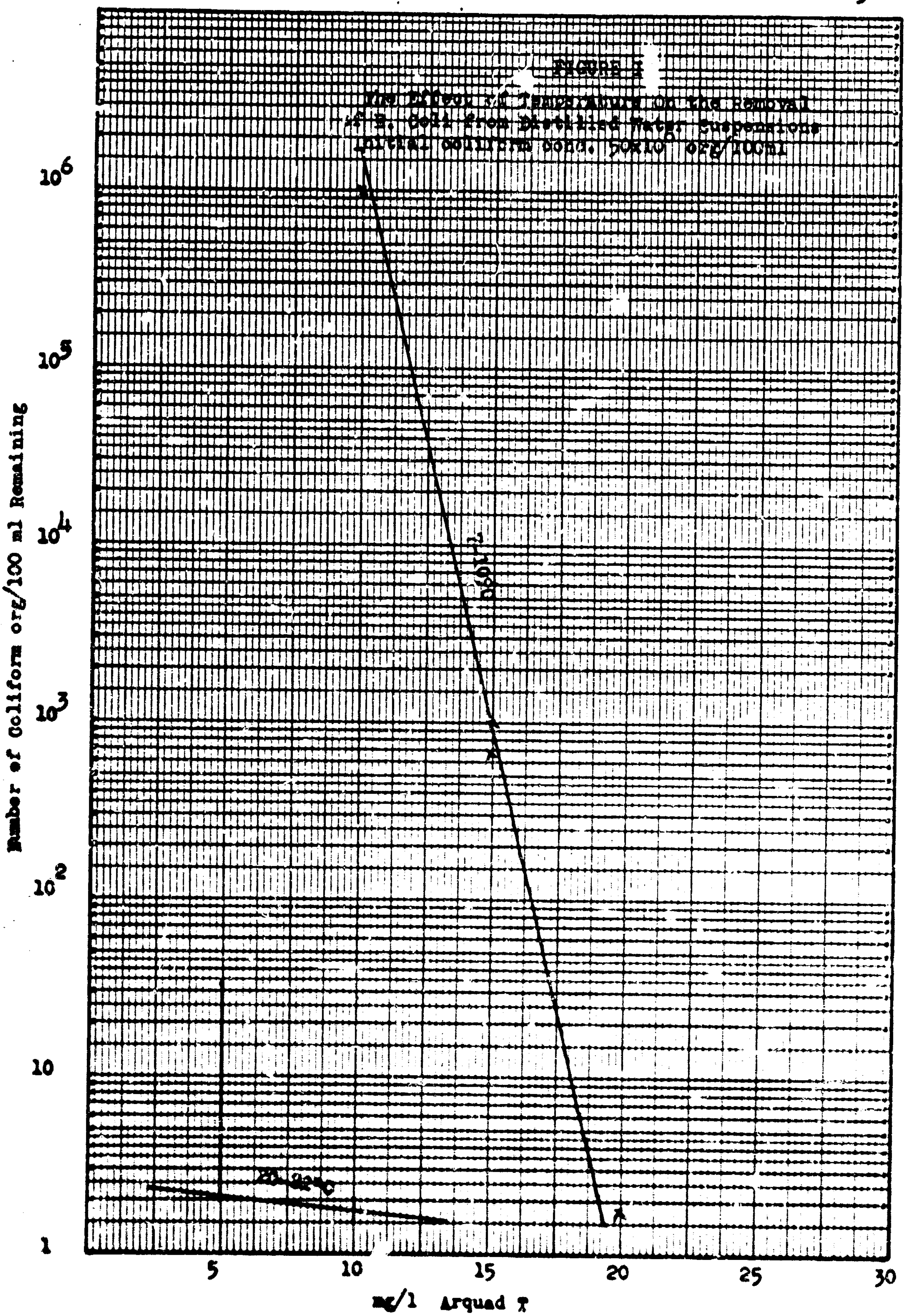
FIGURE II
The Effect of Temperature on the Removal
of E. Coli from Distilled Water Suspensions
Number of Coliform org/100 ml Remaining
10^6
10^5
10^4
10^3
10^2
10
1
5
10
15
20
25
30
mg/1 Arquad T

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A series of experiments were made with an anionic (Nacconol) and a non-ionic (Tween 80) detergent. These experiments showed that there was little or no removal of phage in either case although copious foaming was observed.

Table I

Removal of E. Histolytica Cysts from Suspensions in Distilled Water

			20-23°C		7-10°C	
Compound	Single Treatment	Double Treatment	No. of Experi- ments Run	Complete Removal in	No. of Experi- ments Run	Complete Removal in
Arquad T		5 + 5 mg/l	4	4	1	0
Ceepryn	5 mg/l		1	1	1	0
"	10 mg/l		4	2	-	-
"		5 + 5 mg/l	3	3	1	1
Zephiran		5 + 5 mg/l	2	2	2	2
Emulsept	10 mg/l		1	1	-	-
"		5 + 5 mg/l	3	3	1	1
Hyamine	10 mg/l		1	1	1	0
"		5 + 5 mg/l	2	2	1	1
Bradosol	10 mg/l		1	1	1	1
"		5 + 5 mg/l	2	2	1	1

The complete results have been tabulated on p. 7 of Annual Report dated 31 March 1953, and p. 3 of Annual Report dated 1 April 1954. Figure II illustrates the effect of dosage and temperature on phage removal by Arquad T. The vertical lines represent the range of percentages of the initial number of phage particles remaining after aeration.

VIII. Removal of Turbidity.

Considerable difficulty was encountered in producing representative turbid water. The first attempts utilized dehydrated clays which were suspended in water. Results were erratic and differed greatly with the age of the suspension. It was evident that the charges on the particles were being altered by ion-exchange processes, and that the suspensions were not typical of natural turbid waters.

Satisfactorily stable turbid waters were finally produced by suspending the finely divided portion of bottom sediments of a local stream and a pond. These gave more consistent results in removal experiments, and seemed to behave like natural turbid waters.

Results of experiments on these waters are shown in Table II. In the course of these experiments the pH of the water was adjusted over a fairly wide range. It is comparatively easy to reduce an initial turbidity of 75-100 units to less than 5 units with a single 10 mg/l dose of quaternary, aerated for 10 minutes. The pH value has little or no effect on removal.

Qualitatively, the initial foam produced in the samples appeared clear; the bulk of the turbidity appeared to come over in the middle portions of the foam. There may be a delay in the reaction between the turbidity and the quaternary.

Further tests were run on several naturally turbid waters and removals of turbidity were found to be satisfactory for practical water treatment. U.S.P.H.S. standards were met with doses of 10 mg/l quaternary aerated for 10 minutes.

The data of these tests are given in Table III.

IX. Removal of Color.

Several colored waters were treated by flotation to observe its efficiency in removing color. Results of the experiments appear in Table III in conjunction with the other data, and are brought together in Table IV.

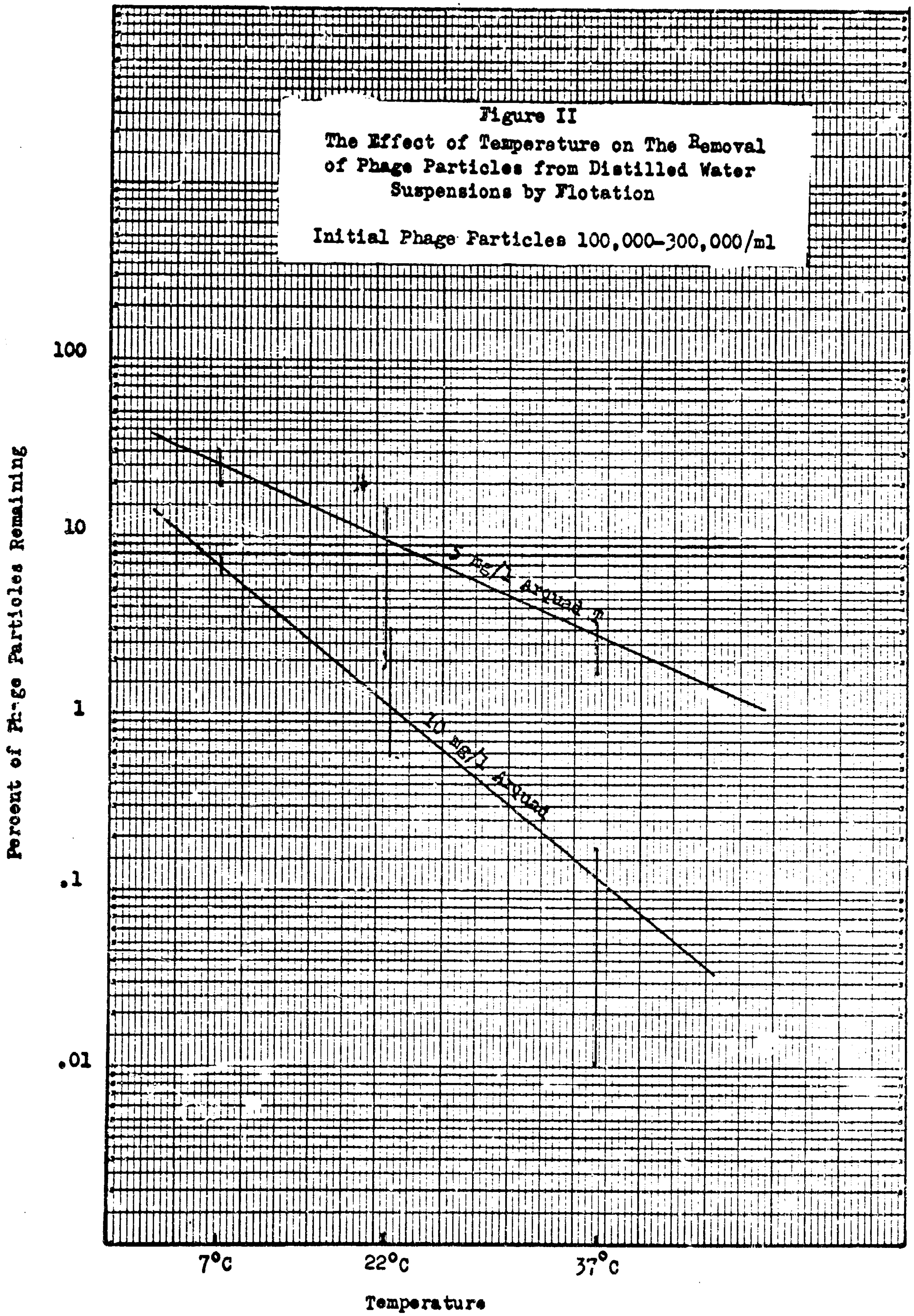


Figure II
The Effect of Temperature on The Removal of Phage Particles from Distilled Water Suspensions by Flotation

Initial Phage Particles 100,000-300,000/ml

TABLE II

Removal of Turbidity from Distilled Water Suspensions of Bottom mud by Arquad T.

Suspension*	Dose of Quatornary	Aeration Time	Initial pH	Initial Turbidity	Final Turbidity	Final pH
A	10 mg/1	10 min.	7.08	100	< 5	7.02
"	"	"	5.27	100	< 5	6.35
"	"	"	3.75	100	< 5	4.12
"	"	"	8.35	100	< 5	8.25
"	"	"	9.07	100	< 5	8.89
"	5	"	7.08	100	10-15	7.06
"	"	"	5.27	100	10-15	6.07
"	"	"	8.35	100	10-15	8.31
"	"	20	7.08	100	10-15	7.04
B	10	10	6.75	75	< 5	6.82
"	"	"	4.85	75	< 5	4.93
"	"	"	8.48	75	< 5	8.26

* Suspension A was a cotton filtered, 24 hr. distilled water suspension of a bottom mud taken from a brook.
Suspension B was a 24 hr. distilled water suspension of a bottom mud taken from a pond in a gravel pit.

Table III

Water from Brook in Lexington, Massachusetts
No additional coliform organisms added.
Quaternary used in flotation, Arquad T.

Dose (mg/l)		10	10	10	15	20
Time of Aeration (min)		5	5	10	5	5
Temperature (°C)		16	13	15	17	15
pH*		6.00	6.80	6.8	6.2	6.7
Alkalinity* (mg/l)		12	24	24	16	24
Hardness* (mg/l)		165	140	140	165	66
Conductivity* mho/cm		$.87\times10^{-3}$	$.7\times10^{-3}$	$.7\times10^{-3}$	$.87\times10^{-3}$	$.25\times10^{-3}$
Turbidity	Initial	5-10 units	15	15	5-10	20
	Final	5 "	< 5	< 5	< 5	< 5
Color	Initial	10 "	30	30	10	120
	Final	< 5 "	5	< 5	< 5	5
Total Bacteria	Initial	25/ml	1800/ml	1800/ml	350/ml	20000/ml
	Final	20/ml	30/ml	10/ml	22/ml	40/ml
Coliform	Initial	3500/100 ml	7000/100 ml	7 000/100 ml	5400/100 ml	92000/100ml
	Final	110/100 ml	220/100 ml	8.1/100 ml	33/100 ml	140/100 ml

* No change during aeration.

(continued on next page)

Table III

Water from Brook in Lexington, Massachusetts
No additional coliform organisms added.
Quaternary used in flotation, Arquad T.

Dose	20	20	10 + 10	10 + 10	30
Time of Aeration	5	10	5 min each	5 min each	5
Temperature	15	17	16	17	15
pH*	6.8	6.8	6.00	6.20	6.7
Alkalinity*	24	24	10	16	24
Hardness*	140	140	165	165	66
Conductivity*	$.7 \times 10^{-3}$	$.7 \times 10^{-3}$	$.87 \times 10^{-3}$	$.89 \times 10^{-3}$	$.25 \times 10^{-3}$
Turbidity Initial	15	15	5-10	5-10	20
Final	< 5	< 5	< 5	< 5	< 5
Color Initial	30	30	10	5	120
Final	< 5	< 5	< 5	< 5	< 5
Total Bacteria Initial	1800/ml	1800/ml	25/ml	350/ml	20000/ml
Final	6/ml	1/ml	14/ml	15/ml	8/ml
Coliform Initial	7000/100 ml	7000/100 ml	3500/100 ml	5400/100 ml	92000/100 ml
Final	170/100 ml	12/100 ml	33/100 ml	49/100 ml	460/100 ml

*No change during aeration.

Table III

Water from Brook in Lexington, Massachusetts
No additional coliform organisms added.
Quaternary used in flotation, Ceepryn.

Dose		10 + 10	20	20	15 + 15	30	30
Time of Aeration		5 min each	5	10	5 min eash	5	10
Temperature*		17°C	15°C	17°C	17°C	15°C	17°C
pH*		6.6	6.7	6.6	6.6	6.7	6.6
Alkalinity*		24	24	24	24	24	24
Hardness*		99	66	90	90	66	90
Conductivity*		$.3 \times 10^{-3}$	$.25 \times 10^{-3}$	$.3 \times 10^{-3}$	$.3 \times 10^{-3}$	$.25 \times 10^{-3}$	$.3 \times 10^{-3}$
Turbidity	Initial	10	20	10	10	20	10
	Final	< 5	5	< 5	< 5	< 5	< 5
Color	Initial	50	120	50	50	120	50
	Final	5	40	< 5	< 5	5	< 5
Total Bacteria	Initial	2000/ml	20,000/ml	2000/ml	2000/ml	20,000/ml	2000/ml
	Final	160/ml	150/ml	70/ml	6/ml	4/ml	4/ml
Coliform	Initial	24,000/ 100 ml	92,000/ 100 ml	24,000/ 100 ml	24,000/ 100 ml	92,000/ 100 ml	24,000/ 100 ml
	Final	700/ 100 ml	6,800/ 100 ml	680/ 100 ml	70/ 100 ml	33/ 100 ml	7.8/ 100 ml

* No change during aeration.

Table III

Water from Brook in Lexington, Massachusetts
Coliform organisms added to raw water.
Quaternary used in flotation, Arquad T.

Dose		10	20	30	15 + 15
Time of Aeration		5	5	5	5 min each
Temperature*		15° C	15° C	17° C	17° C
pH*		6.8	6.8	6.8	6.8
Alkalinity*		24	24	24	24
Hardness*		140	140	154	154
Conductivity*		$.69 \times 10^{-3}$	$.69 \times 10^{-3}$	$.86 \times 10^{-3}$	$.86 \times 10^{-3}$
Turbidity	Initial	10	10	10	10
	Final	5	< 5	< 5	< 5
Color	Initial	30	30	20	20
	Final	5	< 5	< 5	< 5
Total Bacteria	Initial	600,000/ml	600,000/ml	320,000/ml	320,000/ml
	Final	41,000/ml	20,000/ml	140,000/ml	------
Coliform	Initial	600,000/ml	600,000/ml	320,000/ml	320,000/ml
	Final	$>1.6 \times 10^6$/100 ml	1.6×10^6/100 ml	920,000/100 ml	240,000/100 ml

* No change during aeration.

Table III

Water taken from the Charles River, Cambridge, Massachusetts
No coliform organisms added to raw water.
Quaternary used in flotation, Arquad T.

Dose	10	10	10	10
Time of Aeration	5	5	5	10
Temperature*	17	18	15	18
pH *	7.4	8.1	7.0	8.1
Alkalinity *	64	64	80	64
Hardness *	470	680	495	690
Conductivity*	$.35 \times 10^{-3}$	$.4 \times 10^{-3}$	$.37 \times 10^{-3}$	$.4 \times 10^{-3}$
Turbidity Initial	5-10	5-10	5-10	5-10
Turbidity Final	< 5	< 5	5	< 5
Color Initial	35	35	35	35
Color Final	5	5	5	5
Total Bacteria Initial	4000/ml	900/ml	67000/ml	900/ml
Total Bacteria Final	70/ml	60/ml	5000/ml	70/ml
Coliform Initial	5400/100 ml	3100/100 ml	32000/100 ml	3100/100 ml
Coliform Final	2400/100 ml	350/100 ml	10000/100 ml	210/100 ml

* No change during aeration.

(Continued on following page)

Table III

Water taken from the Charles River, Cambridge, Massachusetts

No coliform organisms added to raw water.
Quaternary used in flotation, Arquad T.

Dose		5 + 5	15	20	20
Time of Aeration		5 min each	10	5	5
Temperature*		15	17	18	17
pH*		7.0	7.7	7.4	7.9
Alkalinity*		80	64	80	56
Hardness*		495	630	121	260
Conductivity*		$.37 \times 10^{-3}$	$.54 \times 10^{-3}$	$.64 \times 10^{-3}$	$.2 \times 10^{-3}$
Turbidity	Initial	5-10	5-10	15	25
	Final	< 5	< 5	5	5
Color	Initial	35	35	35	35
	Final	5	< 5	5	5
Total Bacteria	Initial	67,000/ml	2,000/ml	14,000/ml	20,000/ml
	Final	5000/ml	70/ml	120/ml	200/ml
Coliform	Initial	32,000/100	5400/100 ml	4300/100	1200/100 ml
	Final	11,000/100	700/100 ml	10/100	100/100 ml

* No change during aeration.

(Continued on following page)

Table III

Water taken from the Charles River, Cambridge, Massachusetts

No coliform organisms added to raw water.
Quaternary used in flotation, Arquad T.

Dose	10 + 10	10 + 10	10 + 10	10 + 10
Time of Aeration	5 min each	5 min each	5 min each	5 min each
Temperature*	17°C	17°C	17°C	18°C
pH*	7.4	7.8	7.9	7.4
Alkalinity*	64	56	56	80
Hardness*	470	660	260	121
Conductivity*	$.35 \times 10^{-3}$	$.58 \times 10^{-3}$	$.2 \times 10^{-3}$	$.64 \times 10^{-3}$
Turbidity Initial	5-10	5-10	25	15
Turbidity Final	< 5	< 5	5	5
Color Initial	35	35	35	35
Color Final	< 5	5	5	5
Total Bacteria Initial	4000/ml	800/ml	20000/ml	14000/ml
Total Bacteria Final	8/ml	10/ml	180/ml	85/ml
Coliform Initial	5400/100 ml	3500/100 ml	1200/100 ml	4300/100 ml
Coliform Final	350/100 ml	350/100 ml	46/100 ml	240/100 ml

* No change during aeration.

(Continued on following page)

Table III

Water taken from the Charles River, Cambridge, Massachusetts

No coliform organisms added to raw water.
Quaternary used in flotation, Arquad T.

Dose		10 + 10	15 + 15	15 + 15	15 + 15
Time of Aeration		10 min each	5 min each	5 min each	5 min each
Temperature*		17°C	17°C	17°C	18°C
pH*		7.8	7.7	7.9	7.4
Alkalinity*		56	64	56	80
Hardness*		660	620	260	121
Conductivity*		$.58 \times 10^{-3}$	$.54 \times 10^{-3}$	$.2 \times 10^{-3}$	$.64 \times 10^{-3}$
Turbidity	Initial	5-10	5-10	25	15
	Final	< 5	< 5	< 5	5
Color	Initial	35	35	35	35
	Final	5	< 5	< 5	5
Total Bacteria	Initial	800/ml	2000/ml	20000/ml	14000/ml
	Final	15/ml	11/ml	8/ml	85/ml
Coliform	Initial	3500/100 ml	5400/100 ml	1200/100 ml	4300/100 ml
	Final	130/100 ml	1600/100 ml	25/100 ml	15/100 ml

* No change during aeration.

(Continued on following page)

Table III

Water taken from the Charles River, Cambridge, Massachusetts

No coliform organisms added to raw water.
Quaternary used in flotation, Arquad T.

Dose	30	30
Time of Aeration	5	5
Temperature*	18°C	17°C
pH*	7.4	7.9
Alkalinity*	80	56
Hardness*	121	260
Conductivity*	$.64 \times 10^{-3}$	$.2 \times 10^{-3}$
Turbidity Initial	15	25
Final	< 5	< 5
Color Initial	35	35
Final	< 5	< 5
Total Bacteria Initial	14000/ml	20000/ml
Final	10/ml	7/ml
Coliform Initial	4300/100 ml	1200/100 ml
Final	70/100 ml	13/100 ml

* No change during aeration.

Table III

Water taken from Hobbs Brook Reservoir, Lincoln, Massachusetts
With and without added coliform organisms.
Both Ceepryn and Arquad T. used in flotation

Quaternary	Ceepryn	Ceepryn	Ceepryn	Ceepryn	Arquad T	Arquad T
Dose	10	10	20	20	20	10 + 10
Time of Aeration	5	10	5	5	5	5 min each
Temperature* °C	14°C	14°C	15°C	17°C	17°C	17°C
pH*	7.00	7.00	7.00	7.00	7.00	7.00
Alkalinity*	16	16	16	16	16	16
Hardness*	44	44	44	44	44	44
Conductivity*	$.12\times10^{-4}$	$.12\times10^{-4}$	$.12\times10^{-4}$	$.12\times10^{-4}$	$.12\times10^{-4}$	$.12\times10^{-4}$
Turbidity Initial	5	5	5	5	5	5
Turbidity Final	< 5	< 5	< 5	< 5	< 5	< 5
Color Initial	50	50	50	50	50	50
Color Final	15	15	5	< 5	< 5	< 5
Coliform added	0	0	0	$67\times10^6/100$	$67\times10^6/100$	$67\times10^6/100$
Total Bacteria Initial	140/ml	140/ml	140/ml	$67\times10^6/100$	$67\times10^6/100$	$67\times10^6/100$
Total Bacteria Final	15/ml	5/ml	4/ml	------	------	-------
Coliform Initial	33/100	33/100	33/100	$67\times10^6/100$	$67\times10^6/100$	$67\times10^6/100$
Coliform Final	< 1.8/100	11/100	3/100	75/100	350/100	790/100

* No change during aeration.

Table IV

Removal of Color from Water by Flotation

Sample*	Quaternary	Dose of Quaternary	Aeration time	Initial pH	Final pH	Initial Color	Final Color
A	Ceepryn	5 mg/l	5 min	7.00	7.02	55	30
A	Ceepryn	10	5	7.00	7.01	55	15
A	Ceepryn	10	10	7.00	6.90	55	< 5
A	Arquad T	5	5	7.00	7.10	55	35
A	Arquad T	10	5	7.00	7.05	55	20
A	Arquad T	10	10	7.00	7.05	55	< 5
A	Hyamine	10	5	7.00	6.95	55	40
A	Hyamine	10	10	7.00	6.95	55	25
B	Ceepryn	5	5	6.80	6.90	50	10
B	Ceepryn	10	5	6.80	6.95	50	< 5
B	Arquad T	5	5	6.80	7.00	50	15
B	Arquad T	10	5	6.80	7.00	50	< 5
C	Ceepryn	5	5	5.55	6.10	40	25
C	Ceepryn	10	5	5.55	6.10	40	15
C	Ceepryn	10	10	5.55	6.15	40	< 5
C	Arquad T	10	5	5.55	6.20	40	20
C	Arquad T	10	10	5.55	6.15	40	< 5

Sample A Charles River, Cambridge, Massachusetts
B Concord River, Concord, Massachusetts
C From swamp in Lexington, Massachusetts

Removals of color were found to be satisfactory as in the case of turbidity and U.S.P.H.S. standards were met with a corresponding dose except in one case where a very high fresh color, following a severe rain storm, required large amounts of quaternary for satisfactory removal.

An initial color of 50-60 units was reduced to less than 5 units with a dose of 10 mg/l of quaternary aerated for 10 minutes. Again, as in the case of turbidity, pH value has little or no effect on removal.

X. Taste Studies.

Tap water solutions of the various quaternaries were made up and tested for taste by a number of observers. It is evident that there need be no direct taste problem from the amounts of quaternary remaining after aeration provided that aeration continues long enough to remove the bulk of the quaternary added. In no case could observers detect any taste in water containing 5 mg/l or less of quaternary. One out of ten observers could detect a noticeable taste at 10 mg/l while all observers could detect a distinct bitter after-taste at 15 mg/l.

XI. Removals of Organisms from Natural Waters.

Representative natural waters were collected from a polluted river (Charles River at Cambridge), a clean, soft colored brook (Lexington, Massachusetts), and a water supply collection reservoir (Hobbs Brook Reservoir, source of Cambridge, Massachusetts raw water). Removals of the natural content of bacteria and coliform organisms, and of added coliforms were studied. Removals of coliforms and 37°C bacteria from these natural waters were very disappointing when compared to the previous distilled water experiments. In no case were coliform numbers brought down to the U.S.P.H.S. standard of less than 1.8 per 100 ml except in the Hobbs Brook Reservoir water, the initial coliform count of which was only 33 per 100 ml. Doses as high as 30 mg/l of quaternary failed to reduce comparatively small numbers of coliforms even to the 50 per 100 ml specified by the U.S.P.H.S. for water to be treated by chlorination only.

When coliforms were added to these natural waters to bring the initial counts up to those us[illegible] in the distilled water suspensions, results were very unsatisfactory. The data of these experiments appear in Table III.

In the course of the work on natural waters, it was found necessary to adopt the millipore filter method exclusively, for the enumeration of coliforms. MaConkey's agar failed because of interference by the

quaternary, and lactose tube MPN methods became excessively laborious because of the wide range of residual numbers possible. The residual quaternary was neutralized with 500 mg/l of sodium lauryl sulfate prior to filtration through the millipore filter. The millipore filter was not adopted until the results had been checked against lactose broth tube results and found to give reasonable conformance.

XII. Studies on Effect of Water Composition.

It was evident from the results on natural waters that other factors besides low temperature have an adverse effect on the process. The presence of calcium and magnesium salts (hardness) was indicated as one of the interfering factors.

As a quick confirmation of this indication, tests were run on two tap waters: Cambridge, Massachusetts, having a pH of 8.3-8.6, hardness 90-115 mg/l and Boston Metropolitan, pH 7.1, hardness 19-22 mg/l. In general, the coliforms remaining in the soft Metropolitan water were from 100 to 1000-fold fewer than those remaining in the Cambridge water after inoculation with the same number of coliforms, and treatment with the same amount of quaternary at the same temperature. However, at temperatures of 25-26°C the remaining organisms in both waters were too few to maintain this relationship. The data of these tests are given in Table V.

As a further check on this conclusion, studies were made on distilled water to which various calcium and magnesium salts were added, and also on distilled water containing sodium bicarbonate. Sodium bicarbonate in amounts up to 500 mg/l as $CaCO_3$ had no significant effect on the removal of coliforms from water suspensions. Calcium and magnesium salts, on the other hand, were shown to have very adverse effects on coliform removals, as compared to those obtained in distilled water suspensions. For equivalent concentrations in terms of calcium carbonate, the adverse effects were of the same order of magnitude whether the added salt was $Ca(HCO_3)_2$, $CaCl_2$, or $MgCO_3$. Apparently only the positive ion is significant, and Mg^{++} is equivalent in effect to Ca^{++}. Subsequent tests showed that polyvalent anions, such as $SO_4^{=}$ and $PO_4^{\equiv}$, added in form of sodium salts, had no effects on removals, either adverse or favorable. Additions of 360 to 500 mg/l of calcium salts as $CaCO_3$ resulted in final treated waters containing 50,000 to 7,000,000 coliforms per 100 ml, as compared with 0 to 3000 in distilled water. The initial coliform content in these tests was 50 million per 100 ml, and the temperatures were 9°C and 25°C. The higher coliform residuals were obtained at the lower temperatures.

Table V

Tap waters taken from the municipal water supplies of Cambridge, Massachusetts and Boston, Massachusetts with added coliform organisms.* Boston tap water with a pH of 7.1 and hardness of 19-22 mg/l. Cambridge tap water with a pH of 8.3-8.6 and hardness of 90-115 mg/l.

Quaternary	Dose	Time of Aeration	Temperature	Organisms Remaining per/100 ml	
				Cambridge	Boston
Ceepryn	20 mg/l	5 min	14	15×10^4	-------
"	"	"	15	-------	40
Arquad T	"	"	15	1×10^6	1×10^3
"	"	"	16.5	-------	180
"	"	"	18	5.7×10^3	------
Ceepryn	"	"	21	18	------
"	"	"	25	22	------
"	"	"	26	--	4
Arquad T	"	"	21	45	------
"	"	"	23	1,720	210
"	"	"	25	30	0

* Initial concentration of coliform organisms 50×10^6/100 ml to 70×10^6/100 ml.

A series of tests was run to determine quantitatively the effects of temperature and $CaCl_2$. Varying amounts of $CaCl_2$ were added to distilled water and tests were run at two temperature ranges 19-26°C and 9°C. The results of these tests are reported in Table III, p. 10 of Annual Report dated 1 April 1954. They are presented graphically in Figures III and IV. The data are reported and plotted by means of the median value and range of the similar tests run. In conjunction with all experiments, a control was run, which received the same dose of quaternary, but no aeration. These data are reported in Table IV of the same report, and plotted in terms of the median value of similar experiments on Figures III and IV as a dotted line.

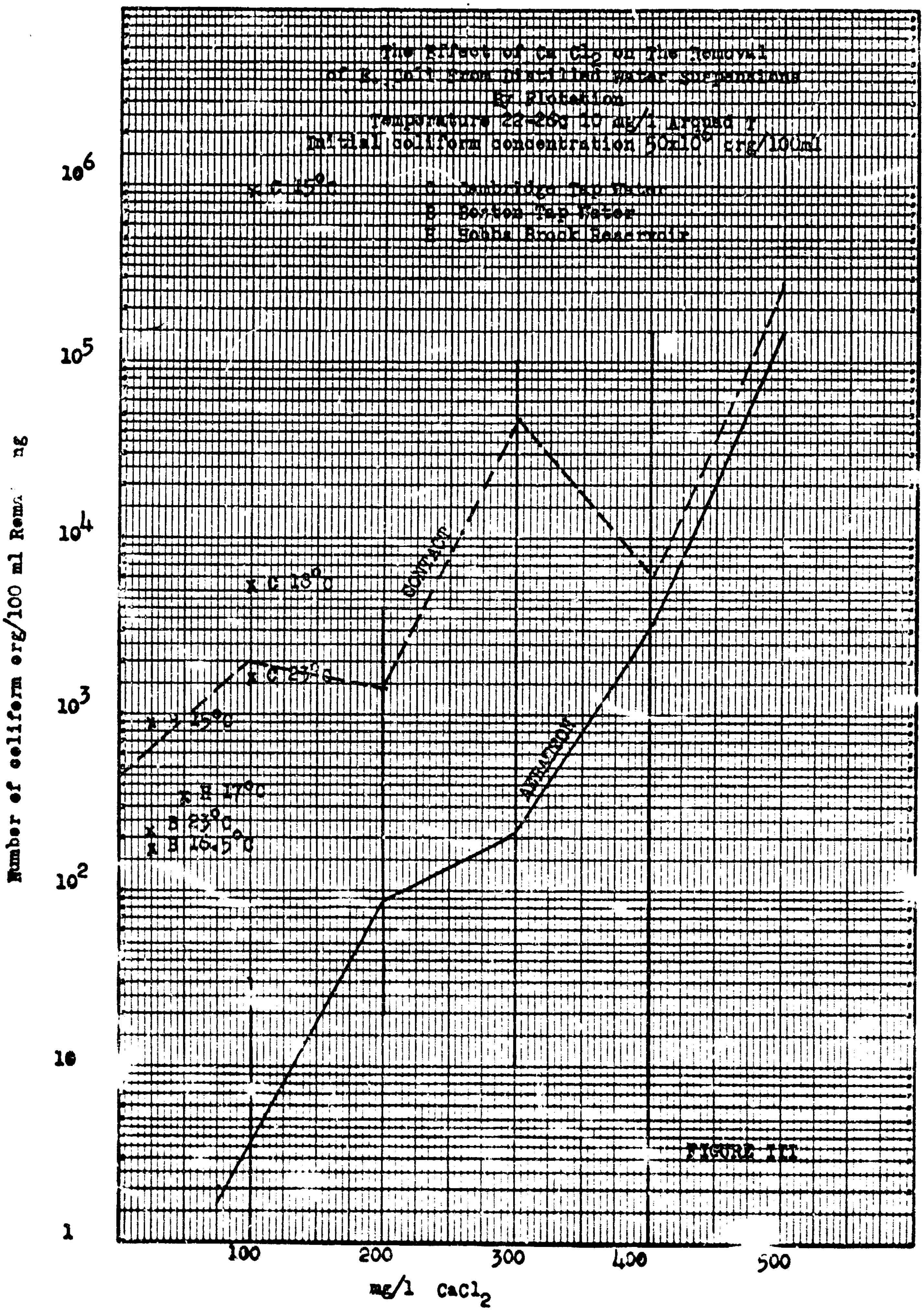
These experiments show that both temperature and $CaCl_2$ content have very large adverse effects. At 19-26°C there is a 10-fold decrease in removal when there is an increase of 100 mg/l of $CaCl_2$. At 9°C there is a 100-fold decrease in removal per 100 mg/l increase of $CaCl_2$.

The similarity of the aerated and contact median lines at 9°C (Figure IV) and the dissimilarity of the aerated and contact median lines at 19-26°C (Figure III) also suggest that the greatest effect of lowered temperature is to interfere with the physical removal of the bacteria by flotation. This is much more affected by temperature than is the rate of killing by the quaternary.

In the light of the above experiments, tests were run to determine the amounts of quaternary required to reduce the coliform numbers in $CaCl_2$ solutions to levels suitable for potable water. These data are reported in Table V, p. 15 of the reported cited above. Figures V and VI show the data.

The work with distilled water showed that 10 mg/l of Arquad T at 20-22°C and 20 mg/l at 7-10°C was sufficient to reduce E. Coli in distilled water suspensions to 1.8 organisms or less per 100 ml. Extrapolation in Figures V and VI would indicate that under the same experimental conditions 300 mg/l of $CaCl_2$ raises the quaternary requirement to 40 mg/l at 24°C and 70 mg/l at 9°C, and 500 mg/l of $CaCl_2$ raises the quaternary requirement to 60 mg/l at 24°C and 130 mg/l at 9°C.

Since the natural waters tested were more difficult to treat than would be predicted by hardness alone, (see Figures III and IV) a series of tests was made to determine the effect of turbidity in the presence of Ca^{++}. Using bottom mud suspensions prepared in the same way as the samples used to study turbidity removal (Section VIII) samples were made up containing various concentrations of turbidity in both pure distilled water and distilled water containing 500 mg/l $CaCl_2$. The results, given in Table VI of the Annual Report dated 1 April 1954 and shown in part on Figure VII indicate that while turbidity has no effect on the efficiency of removal in samples containing no $CaCl_2$, the combination of $CaCl_2$ and turbidity has a greater adverse effect on the efficiency of removal than $CaCl_2$ alone.



The Effect of Ca Cl2 on The Removal
of E. Coli from Distilled water suspensions
By Flotation
Initial coliform concentration
C Cambridge Tap Water
B Boston Tap Water
H Hobbs Brook Reservoir
x C 13°C
x C 23°C
x H 17°C
CONTACT
AERATION
FIGURE III
10^6
10^5
10^4
10^3
10^2
10
1
100
200
300
400
500
mg/l $CaCl_2$
Number of coliform org/100 ml Rem

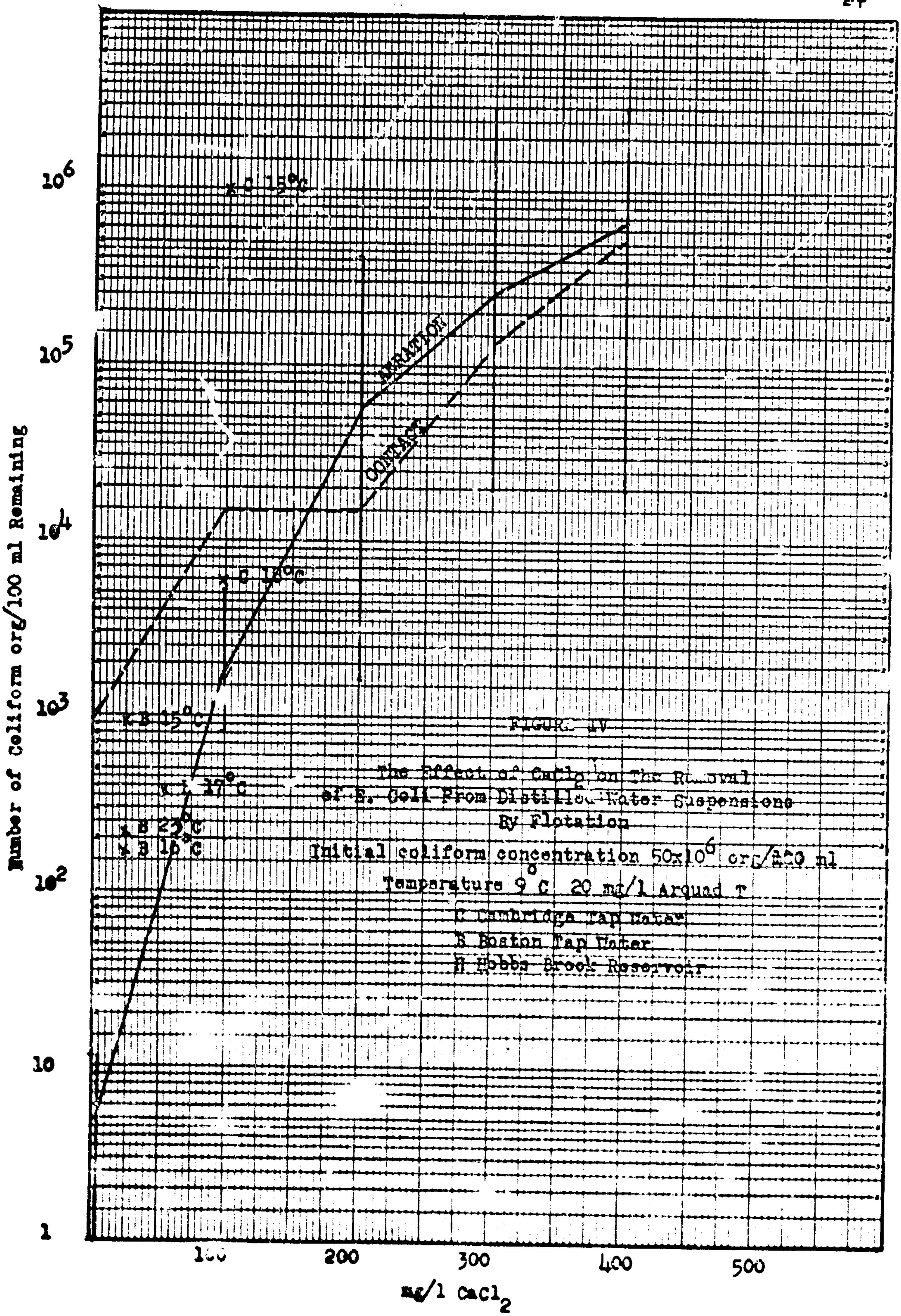


FIGURE IV

The Effect of $CaCl_2$ on The Removal of E. Coli From Distilled Water Suspensions By Flotation

Initial coliform concentration 50×10^6 org/100 ml

Temperature 9°C 20 mg/l Arquad T

C Cambridge Tap Water

B Boston Tap Water

H Hobbs Brook Reservoir

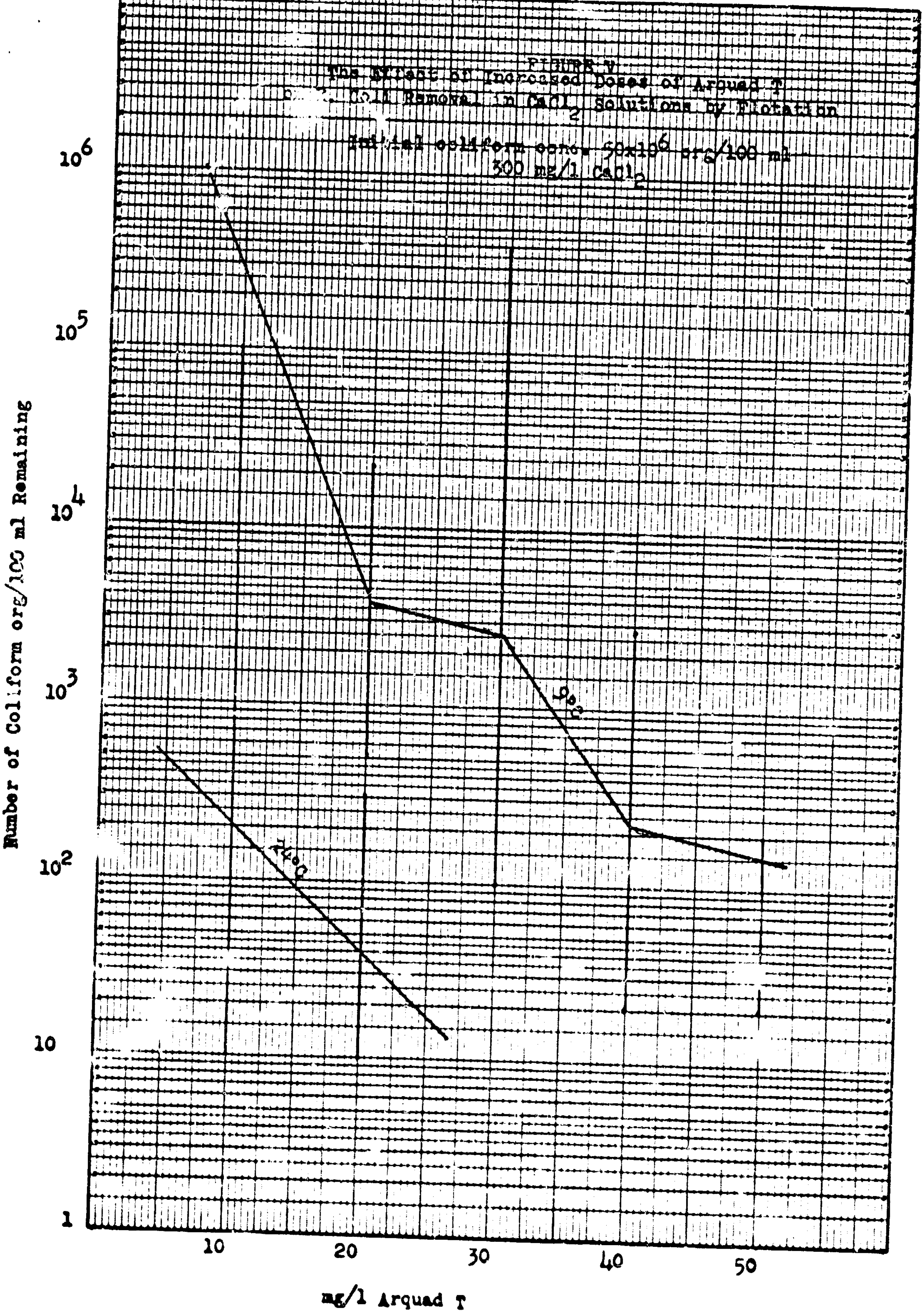
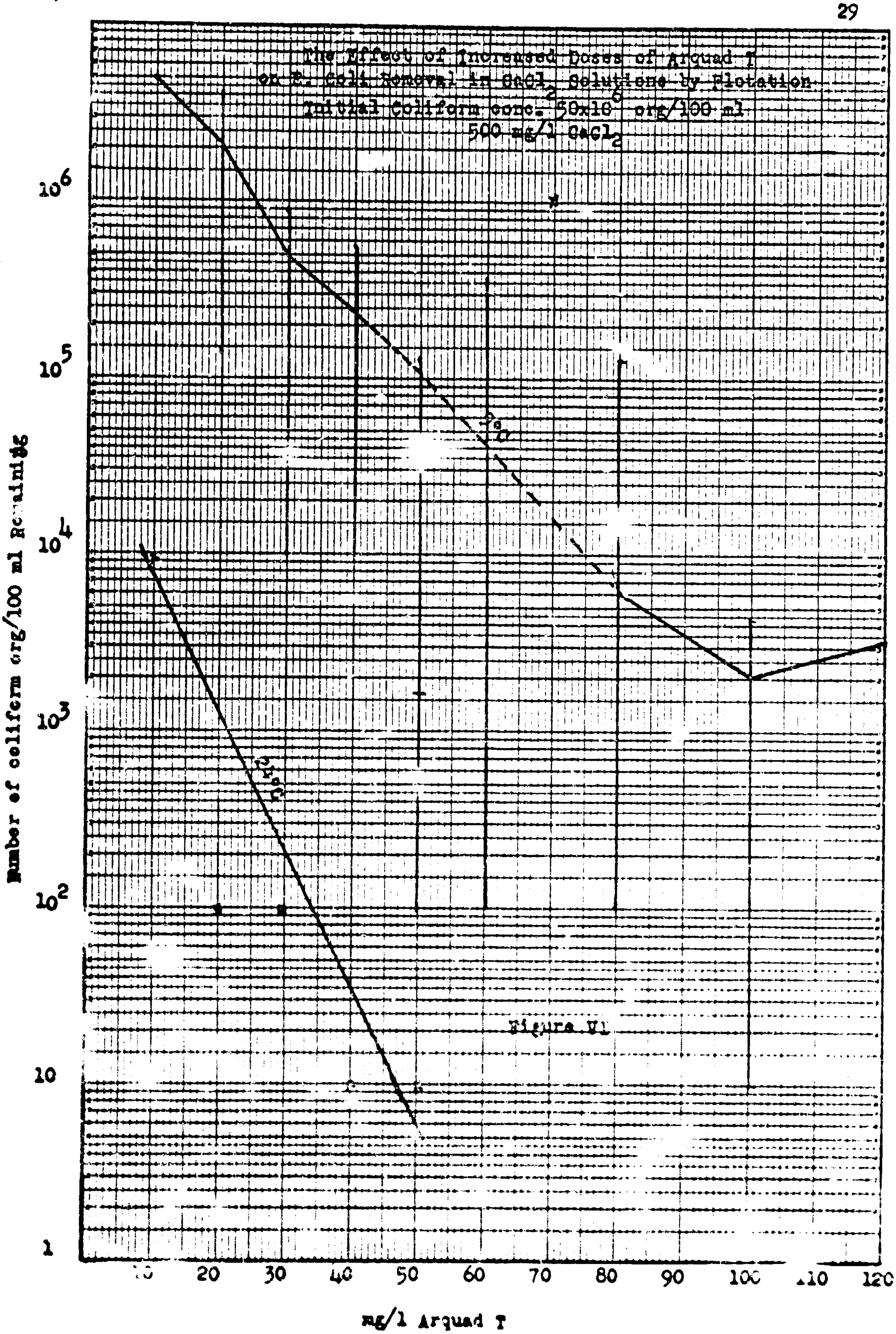


FIGURE V
The Effect of Increased Doses of Arquad T
Coli Removal in CaCl2 Solutions by Flotation
coliform conc. 50x10^6 org/100 ml
300 mg/1 CaCl2
Number of Coliform org/100 ml Remaining
10^6
10^5
10^4
10^3
10^2
10
1
10
20
30
40
50
mg/1 Arquad T


The Effect of Increased Doses of Arquad T
on E. Coli Removal in CaCl$_2$ Solutions by Flotation
Initial Coliform conc. 50x10^6 org/100 ml
500 mg/l CaCl$_2$
Number of coliform org/100 ml Remaining
10^6
10^5
10^4
10^3
10^2
10
1
20
30
40
50
60
70
80
90
100
110
120
mg/l Arquad T
Figure VI

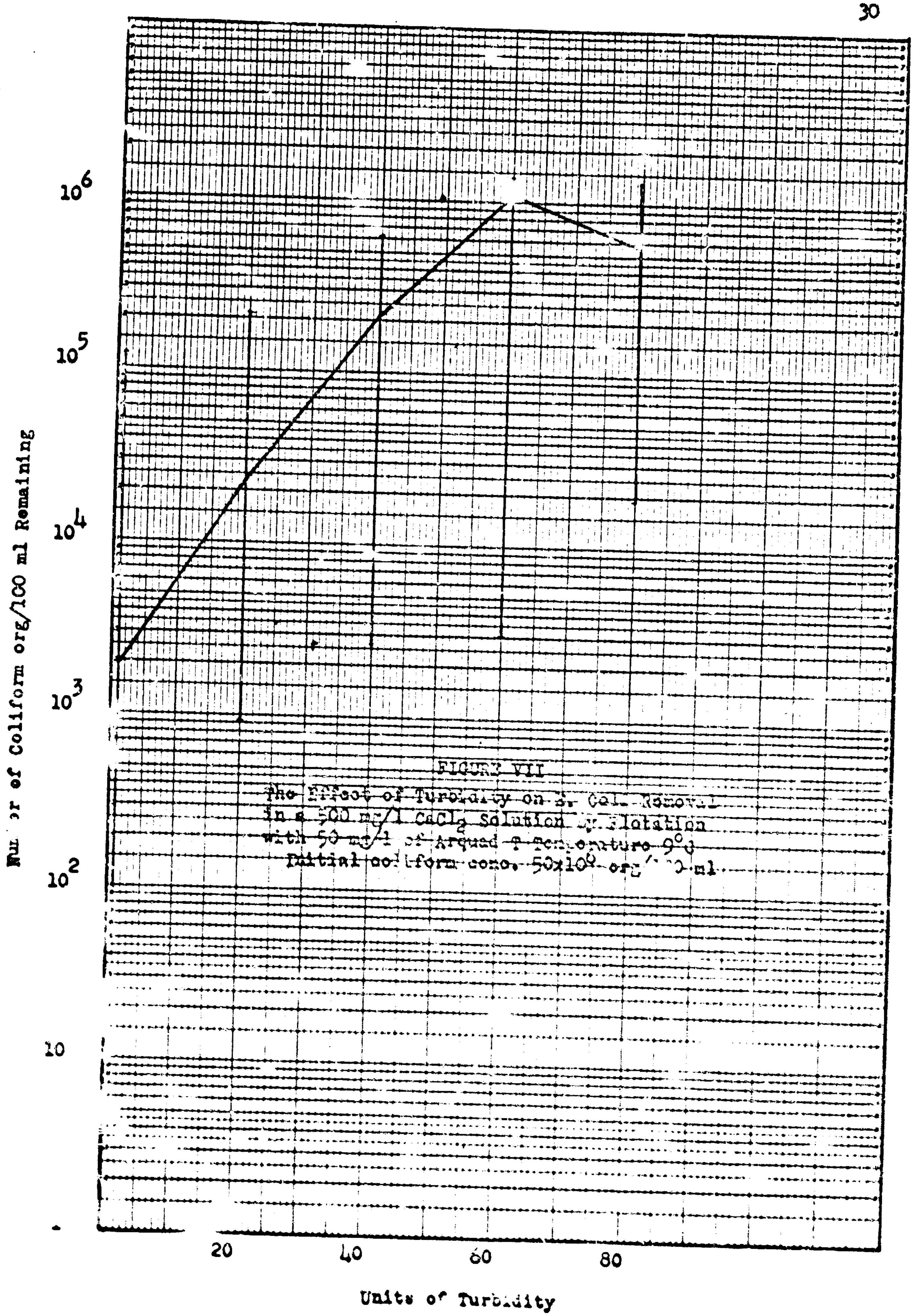


FIGURE VII

The Effect of Turbidity on E. Coli Removal in a 500 mg/l $CaCl_2$ Solution by Flotation with 50 mg/l of Arquad T Temperature 9°C

Initial coliform conc. 50×10^6 org/100 ml

The electrophoretic studies described in a later section of this report indicate that color would also show an additive adverse effect, like that shown by turbidity.

In the light of the adverse effect on removals produced by the divalent positive ions Ca^{++} and Mg^{++}, an investigation was made of the effect of a trivalent ion, Al^{+++}, which may occur in natural waters. The adverse effect of this ion was found to be very large. It was found that 0.1 mg/l of Al^{+++} (as $CaCO_3$) had about the same adverse effect as 500 mg/l of Ca^{++} (as $CaCO_3$). No more than 90% of the 50×10^6 coliforms initially present in 100 ml of a solution containing as little as 0.5 mg/l of Al^{+++} (as $CaCO_3$) could be removed, even with as much as 50 mg/l of Arquad T.

The data of the Al^{+++} experiments appear in Table VI, which shows the median values and ranges of results at various Al^{+++} concentrations. Results of concentrations from 0 to 1 mg/l are shown in Figure VIII. The observed differences at concentrations above this are not too significant but the adverse effect unexpectedly becomes somewhat smaller at very large concentrations of Al^{+++}.

The presumption is that other trivalent positive ions, - for example, Fe^{+++} - might have similar effects.

It should be noted that in the experiments on Al^{+++} the aeration rate was reduced to 0.72 liters per liter per minute, and the time increased to 10 minutes, to try to secure the benefits of increased efficiency noted in the next section.

Table VI

The Effect of Al^{+++} on the Removal of E. Coli from Distilled Water Suspensions by Flotation

Initial concentration of coliform organisms 50×10^6/100 ml.
Temperature [illegible]°C 50 mg/1 Arquad T.
Aeration Rate .72 1/1/min for 10 minutes

mg/1 $AlCl_3$ as $CaCO_3$ equivalent	Organisms per 100 ml left after aeration: Median	Organisms per 100 ml left after aeration: Range
300	245,000	240,000 - 250,000
200	1,500,000	510,000 - 1,800,000
150	1,250,000	940,000 - 1,730,000
100	1,300,000	1,020,000 - 1,500,000
50	8,000,000	[illegible]0,000 - 8,000,000
40	6,000,000	6,000,000 - 9,000,000
30	7,000,000	5,000,000 -10,000,000
20	6,500,000	6,000,000 -10,000,000
10	400,000	400,000
5	9,700,000	9,700,000
4	10,000,000	4,000,000 -10,000,000
3	11,000,000	10,000,000 -11,000,000
2	8,500,000	8,500,000
1	4,000,000	2,500,000 - 9,000,000
0.5	7,500,000	2,000,000 -12,250,000
0.4	7,000,000	1,700,000 - 9,250,000
0.3	4,500,000	150,000 - 9,000,000
0.2	10,000	10 -10,000
0.1	10,000	10 -10,000
.08	60	40 - 80
.06	16	2 - 40
.04	2	0 - 8
.02	30	18 - 40
.01	[illegible]	21 - 48
0	0	0 - 6

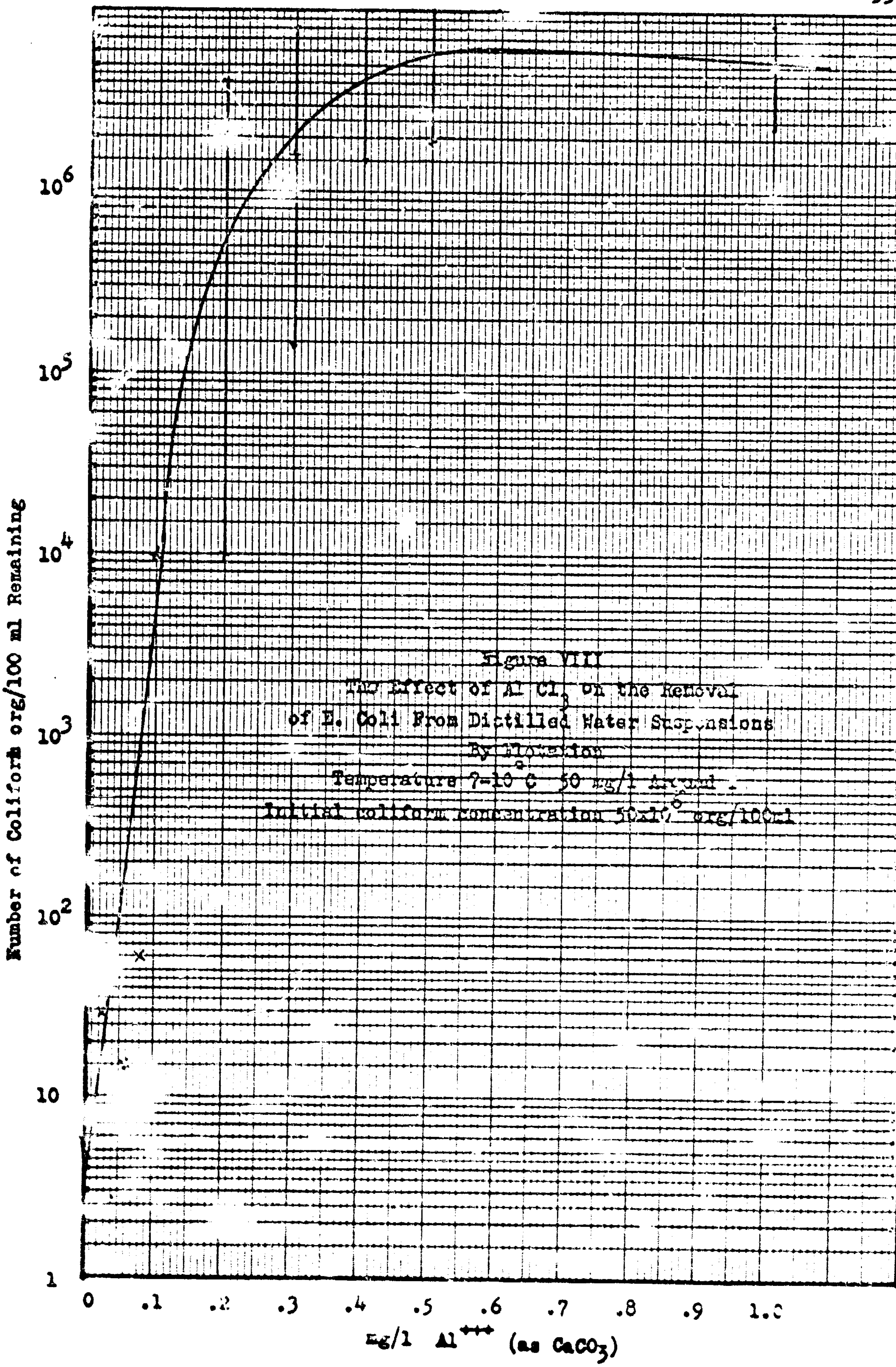


Figure VIII
The Effect of $Al\ Cl_3$ on the Removal
of E. Coli From Distilled Water Suspensions
By Flotation
Temperature 7-10°C 50 mg/l Arquad
Initial coliform concentration 50×10^6 org/100ml

XIII. Air Rates and Air Quantities.

Following the practice of Hopper, et al (JAWWA 44, 719 (1952), an aeration rate of one liter of air per liter of suspension per minute was used for most experiments. The period of aeration at this rate was 5 or 10 minutes. This rate was satisfactory as to foaming characteristics and percent of water lost in the foam as long as quaternary dosages remained in the 5 to 10 mg/l range. About 1% of the water in the suspension went over in the foam, for each mg/l of quaternary added, at the one liter/liter/minute rate.

When the adverse factors of natural waters required advances in quaternary dosage to 30, 40, or 50 mg/l, water losses became very large. It was found that reduction in aeration rates to 0.72 or 0.54 liter per liter per minute, and extensions of aeration times to 15 or 20 minutes produced very small water losses, and much greater efficiencies of bacterial removal in distilled water suspensions containing large amounts of Ca^{++}. This data appeared on p. 20 of the Annual Report dated 1 April 1954.

However, this hopeful lead was negated by subsequent observations:

(1) No such great improvements in efficiency were observed in the experiments on waters containing Al^{+++}.

(2) In many natural waters, the low aeration rates failed to produce any foam at all, and therefore no removal of organisms, unless the quaternary dose was at least 30 mg/l.

(3) Much greater quantities of air were required at the low rates, to reduce the residual quaternary in the treated water to desirable levels, as compared with the quantities required at one liter of air per liter per minute. If the volume of air required at one liter per liter per minute was x, that required at 0.72 liter per liter per minute was approximately 2x, and at 0.54 liter, 3x. The residual quaternary was estimated by observing the foaming characteristics.

XIV. Studies on Electrophoretic Migration of Bacteria.

Investigation of the migration of coliform organisms in an electric field, both before and after treatment with quaternary, was undertaken in the hope that such studies would give some indication of the nature of the interferences by polyvalent cations, color, and other components of natural waters. The equipment used was a Northrup-Kunitz electrophoresis apparatus; the individual coliform organisms were observed with an ordinary microscope using the 4 mm objective. A battery and potentiometer provided a constant potential gradient of 0.035 volts per millimeter. The observations, taken at the proper depth to reduce electro-osmotic flow to a minimum, consisted of measuring the time for the organisms to move 0.5 millimeters along an ocular micrometer. The reciprocal of this time is the velocity of the organism, which is proportional to its zeta potential, if other conditions are maintained approximately the same.

These observations are presented graphically in Figures IX, X, XI, XII, and XIII. In distilled water, prior to the addition of quaternary, E. Coli showed a relatively high negative charge, as observed by other workers. Progressively increasing doses of quaternary reduced this negative charge; the charge became zero, and subsequently positive, as the doses were further increased. Since measurements of this type are only approximate, because of interference by electro-osmotic currents, smooth curves of velocity against quaternary dose are not obtained, but the results are sufficiently precise to establish trends.

E. Coli in distilled water requires but little quaternary to alter its charge; the charge becomes zero when about 3 mg/l of quaternary is added, and rises to a high positive value at quaternary doses of 8 to 10 mg/l. (The term "high" is used only relative to the initial negative charge.) Evidently fairly large amounts of quaternary are picked up rapidly, possibly by some type of adsorption or ion exchange mechanism.

In general, the effect of the components previously found to interfere with removal of organisms by flotation is to greatly increase the dosage required to produce a given change in charge, and to greatly decrease the positive values of charge attained at the high dosages. This is made very evident by comparing the distilled water curves and the other curves in Figures IX, X, XI, XII, and XIII.

In the presence of 100 mg/l or more of Ca^{++}, - as appears in Figure IX - about 4 to 6 mg/l of quaternary is required to bring the bacterial charge to zero, and 30 mg/l to increase it to the positive value reached in the distilled water suspensions at 10 mg/l of quaternary. Figure X shows that very small quantities of Al^{+++} have large effects. In the presence of 0.06 mg/l of Al^{+++} (as $CaCO_3$), 6 mg/l of quaternary are needed to give zero bacterial charge, and 18 mg/l of quaternary gives the same positive charge as 8 mg/l in distilled water. This confirms the previous observation that 0.1 mg/l of Al^{+++} has as great an adverse effect as 500 mg/l of Ca^{++} on removal of coliform organisms by flotation. (Section XII)

Color, either natural or added, has similar effects; presumably other organic components would do the same. Figure XI shows what happens when a simulant of natural color, - stale tea - is added to distilled water, and to a filtered tap water (Cambridge, Massachusetts). In either case, the quaternary requirement for equivalent change of bacterial charge was greatly increased, and the ultimate positive charge attained greatly decreased. An added color equivalent to 200 units of natural color doubles quaternary requirements in the tap water, and increases them by seven fold in the distilled water.

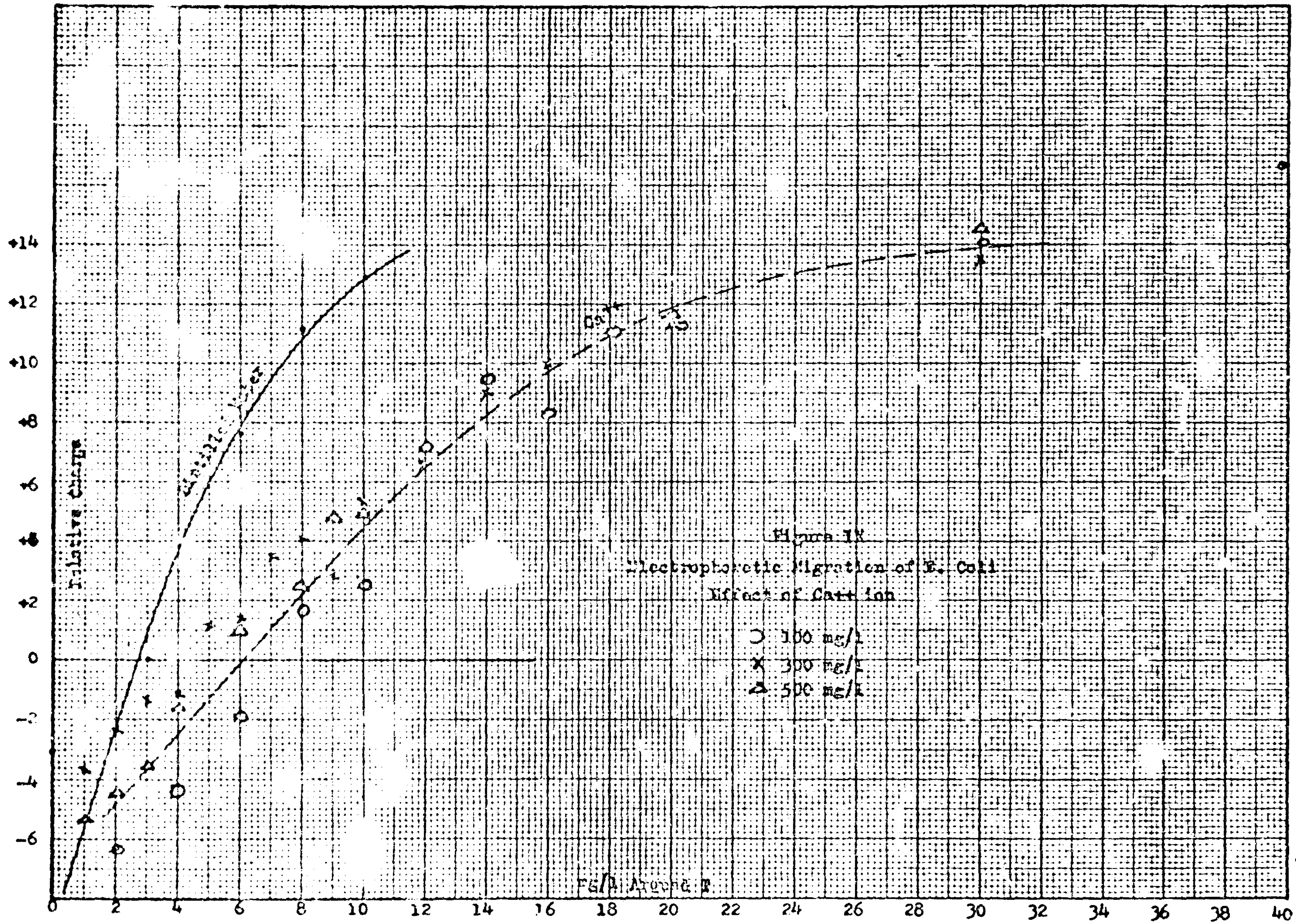


Figure IX
Electrophoretic Migration of E. Coli
Effect of Ca++ Ion
100 mg/l
300 mg/l
500 mg/l
Relative Charge
Distilled Water
mg/l Around F
+14
+12
+10
+8
+6
+4
+2
0
-2
-4
-6
0
2
4
6
8
10
12
14
16
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20
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28
30
32
34
36
38
40

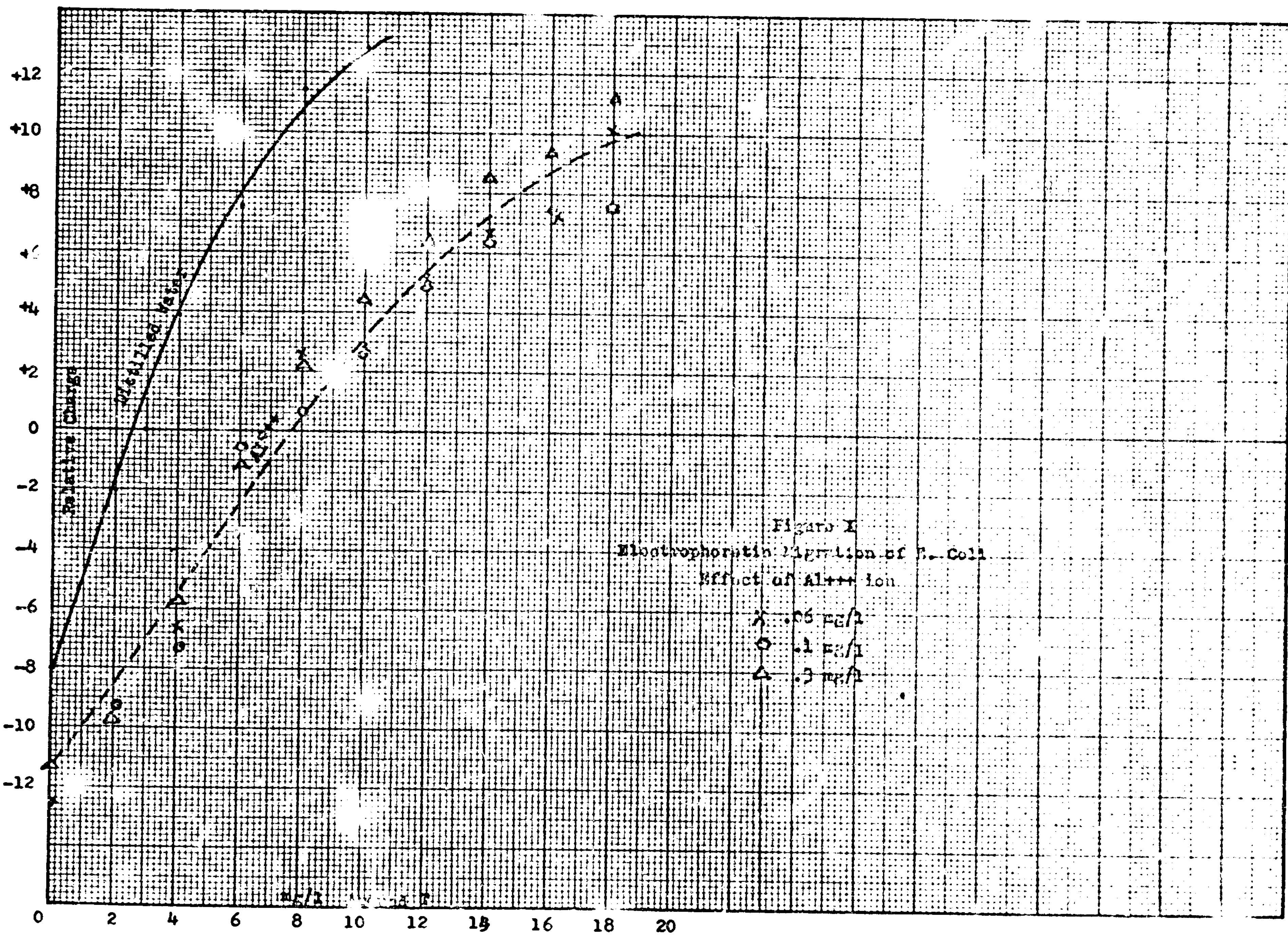

Figure X
Electrophoretic Migration of E. Coli
Effect of Al+++ Ion

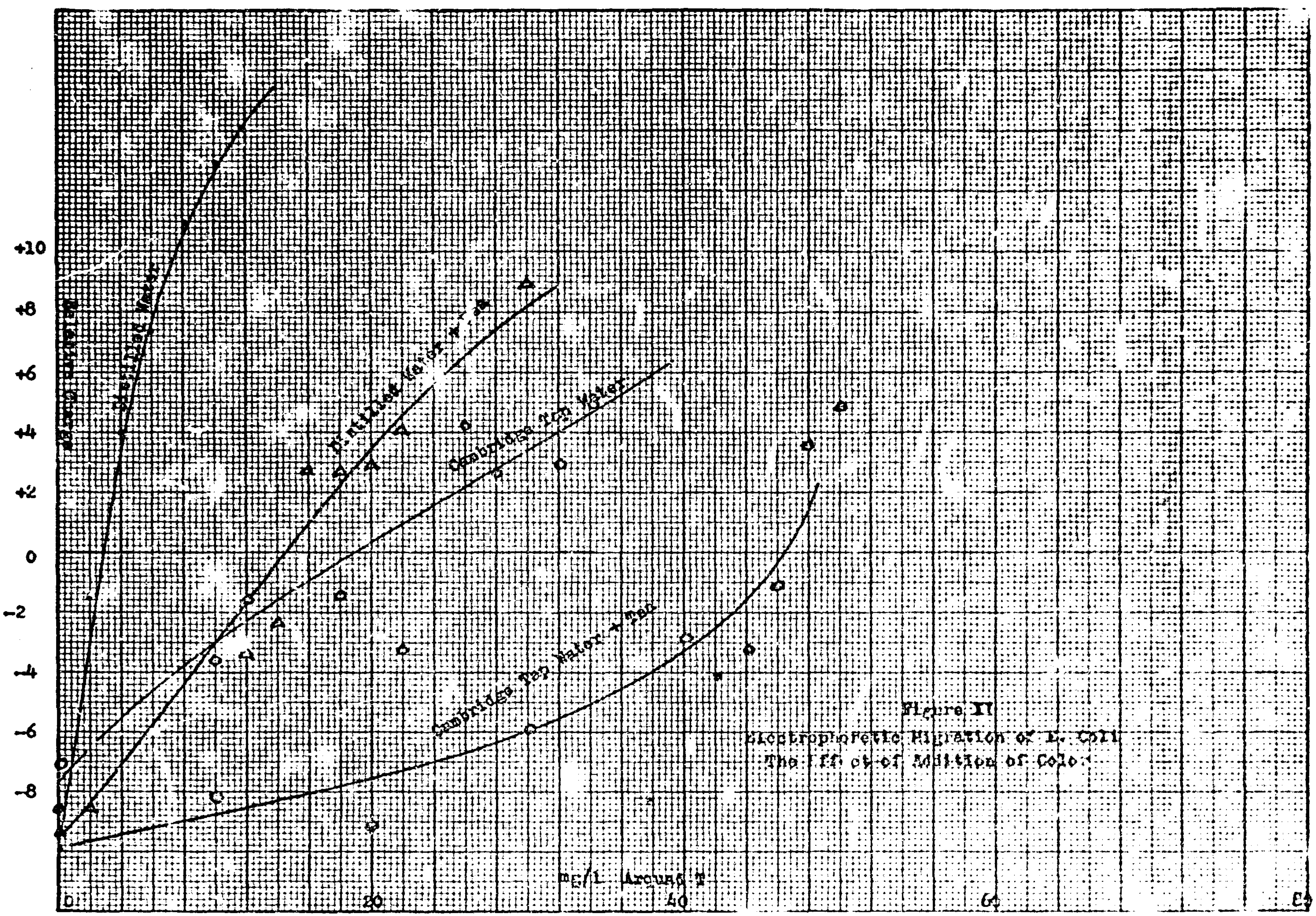

Figure XI
Electrophoretic Migration of E. Coli
The Effect of Addition of Colo·

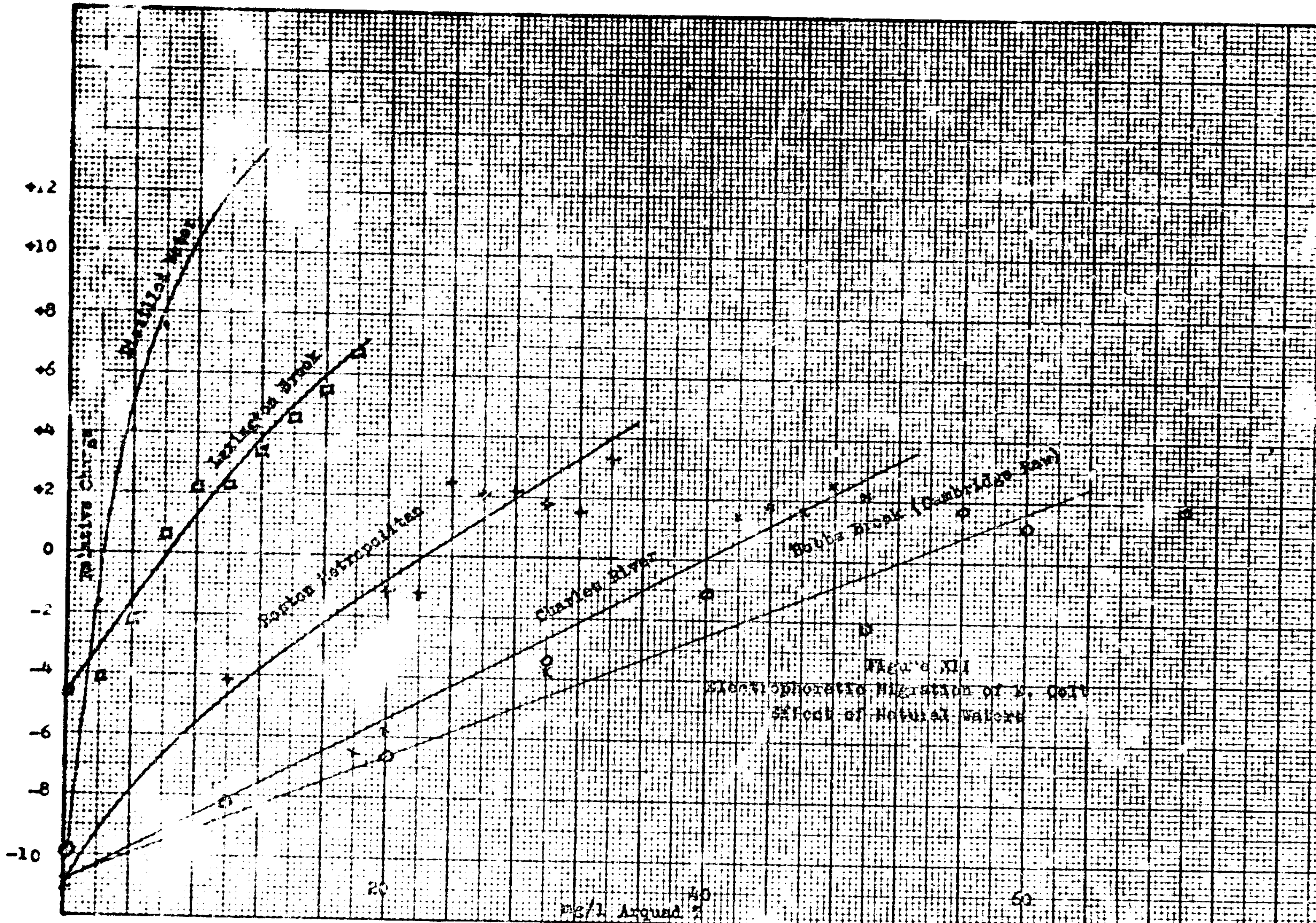

Figure XII
Electrophoretic Migration of E. Coli
Effect of Natural Waters

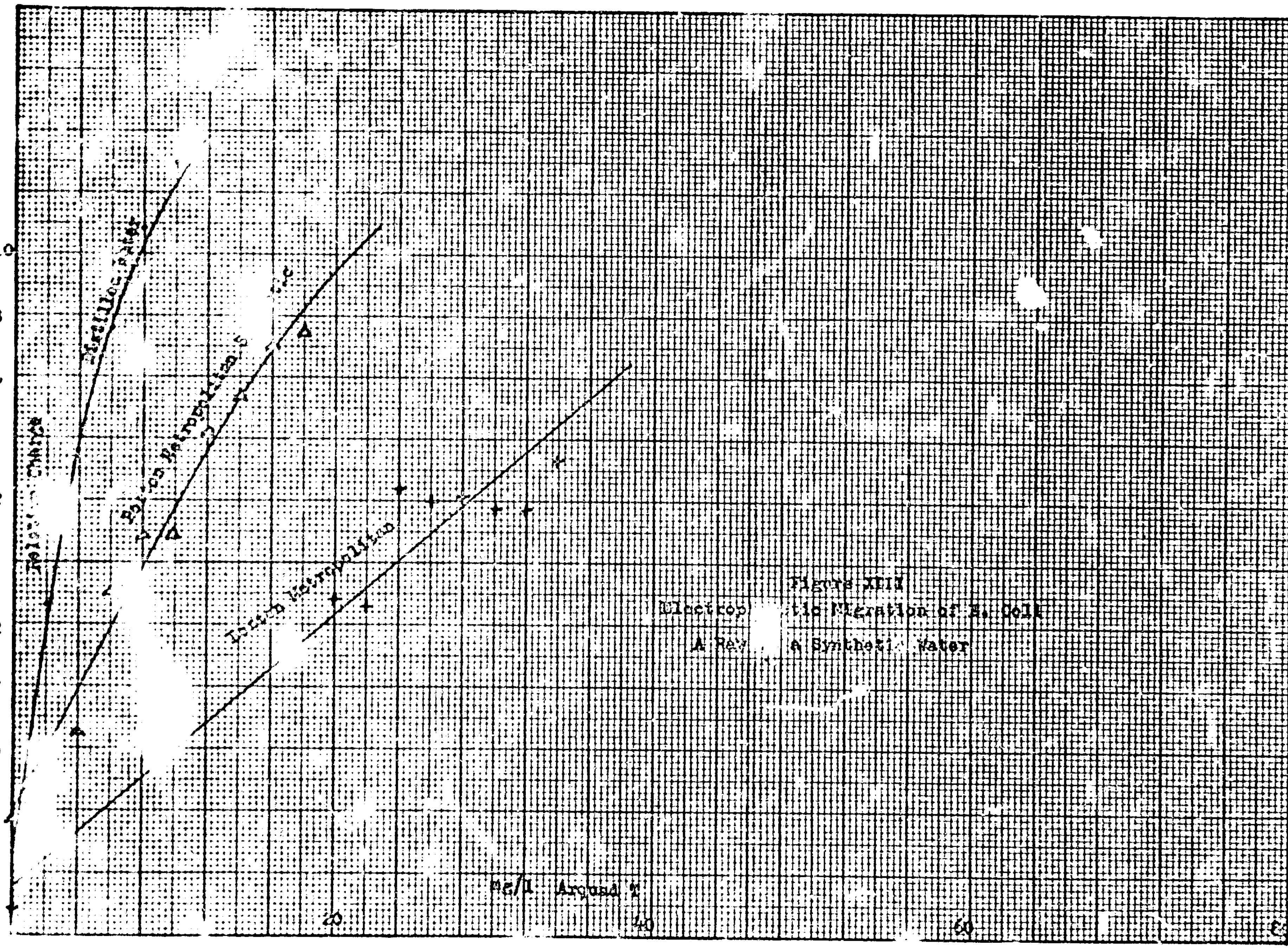

Figure XIII
Electrop tic Migration of E. Coli
A Rev a Synthetic Water

In natural waters, the effects of the interfering components are more or less additive, as shown in Figure XII. The Lexington Brook, a soft water of moderate color, comes closest to distilled. Charles River, with about the same color content, but a much higher hardness, and a considerable degree of pollution has a far greater quaternary requirement, as does also the Hobbs Brook Reservoir water (Cambridge raw water) which is soft but highly colored. It is interesting to compare this with the final Cambridge tap water, which is increased in hardness, but substantially freed of color. The treatment lowers quaternary requirements to get to zero bacterial charge from 50 mg/1 to 22 mg/1.

The position of Boston Metropolitan tap water is somewhat anomalous; it is an unfiltered water low in hardness and color, but its quaternary requirements are greater than would be expected. Typical analyses of these waters are given in Table III. Figure XIII shows a comparison between this water and a synthetic water made up to contain all the identified mineral constituents of the tap water. The synthetic water has much much lower quaternary requirements than the natural tap water, and, in fact, closely approaches distilled water. It is evident that small quantities of unidentified components, such as organic matter, silica or polyvalent positive ions must account for the difference. This is an example of the extreme sensitiveness to small quantities of certain components.

Conclusions from Electrophoretic Studies.

The results of the electrophoretic studies are in line with those obtained in the studies of the influence of water composition on removal of organisms by flotation. They indicate that several components found in natural waters, - some identified, some not - interfere greatly with the sorption of quaternary ammonium compounds by bacteria. If the quaternary is not sorbed by the bacterium, presumably it can neither kill the organism nor aid in its attachment to the film of an air bubble for mechanical removal in the froth.

These experiments do not elucidate the mechanism of the interference; it may either be a mod tion of the surface of the bacterium so that less quaternary is adsorbed for a given concentration in the water, or it may be a modification or adsorption of the quaternary itself by the interfering agent, so that less of the added quaternary is available for the bacterium. Either mechanism will explain the observation t the charge on the organism can still be changed by increasing the amounts of quaternary. In any case, the process is indicated as being very sensitive to natural water composition.

XIV. General Conclusion.

The original proposal envisioned the purification of water by removing color, turbidity, and micro-organisms in the film of bubbles formed by adding quaternary ammonium compounds to the water and aerating, taking off the froth of bubbles as formed. This procedure appeared to offer several advantages, among them the elimination of cumbersome filters, the speeding up of the purification procedure, and a reduction in the actual bulk of chemicals used in purification. In view of these possible advantages, it was given consideration as a possible field purification method for the armed forces.

The results of this study have shown that this method is indeed capable of a high degree of removal of micro-organisms ranging from amoebic cysts, through bacteria to viruses and also a high degree of removal of such aesthetically objectionable features as color and turbidity. This removal is conditional on the addition of sufficient quaternary ammonium compound, and aeration at a slow enough rate, for a long enough time, to ensure removal, while at the same time keeping loss of water in the foam at a minimum.

The major difficulty with the process is implied in the preceding sentence. Low temperatures, and a number of components present in natural waters, - of which the hardness ions, some polyvalent ions, such as Al^{+++} color, and turbidity have been identified, - all increase the amount of quaternary required to secure adequate removal. This increase further entails a slowing of aeration rates and a lengthening of aeration time, to secure removal of the quaternary (avoidance of taste) and avoidance of excessive water loss in the foam.

The alternatives in practical use would seem to be either (a) to have a fairly complete analysis of the raw water to be treated in order to estimate the required dose of quaternary, or (b) to set the quaternary dose and aeration time and rate at levels adequate to cope with any variation in water composition likely to be encountered. This latter one would seem to be the only possible procedure in the field.

In view of the marked effects, for example, of even very small quantities of Al^{+++}, it would seem that setting arbitrary figures high enough for all waters that could be encountered in the field would be both uneconomic and precarious. It is therefore concluded that unless further research indicates ways of overcoming the interference, the process is not adapted to field use. To date, no leads have been found on overcoming the interference. This conclusion does not preclude consideration of the process where water composition is known and fairly stable; for this application it has some interesting potentialities.